Influence of the Common Human δ-Aminolevulinate Dehydratase Polymorphism on Lead Body Burden

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δ-Aminolevulinate dehydratase (ALAD) is the second enzyme in the heme biosynthesis pathway. ALAD is a zinc metalloenzyme, and its inhibition by lead substitution for zinc is one of the most sensitive indicators of blood-lead accumulation, a measure of recent lead exposure. Stoichiometry calculations indicate that a significant portion of blood lead is stored in ALAD. Human ALAD exhibits a charge polymorphism, with about 20% of Caucasians expressing the rarer $ALAD^2$ allele. Human $ALAD^1$ and $ALAD^2$ cDNAs and the 16-kb ALAD gene have been cloned and sequenced. A simple polymerase chain reaction test has been established and validated for determining ALAD genotypes. Two population studies have indicated that lead-exposed individuals with the $ALAD^2$ allele have blood-lead levels about 10 μg/dl greater than similarly exposed individuals carrying only the $ALAD^1$ allele. Ongoing work is directed toward determining the biochemistry underlying the allele-specific accumulation of blood lead, and toward determining the contribution of human ALAD genotype to lead accumulation in other tissues in transgenic mouse models and to final lead deposition in bone in both mouse and man. — Environ Health Perspect 102(Suppl 3):215–219 (1994)

Key words: lead, δ-aminolevulinate dehydratase, polymorphism, blood, human, zinc, metalloenzymes

Introduction

Exposure to environmental lead may affect numerous organ systems, including the renal, reticuloendothelial, reproductive, and nervous systems. Of particular importance, exposure to low levels of lead *in utero* or in early childhood may lead to irreversible central nervous system damage (*1–8*). Although the reduced use of leaded gasoline, widespread detection of lead-based paint poisoning of children, and control of lead exposure in the workplace have combined to substantially reduce the incidence of acute lead poisoning in the United States, exposure to low doses of lead is still quite common. The Second National Health and Nutrition Examination Survey (NHANES-II) found that 1.5 million preschool children had blood lead levels of 25 μg/dl and above, indicating significant lead absorption.

The homooctameric zinc metalloenzyme, δ-aminolevulinate dehydratase (porphobilinogen synthase, ALAD, EC 4.2.1.24), is the second enzyme in the heme biosynthetic pathway. ALAD is expressed in all tissues, but occurs at elevated levels in liver, which has the added burden of cytochrome P450 synthesis, and in the highest levels in erythrocytes and their precursors. ALAD is stoichiometrically inhibited by lead (*9–11*), with lead replacing zinc. Erythrocyte ALAD inhibition has been used as a routine lead-poisoning test in many clinical chemistry laboratories.

Lead inactivation of ALAD has been implicated in the pathogenesis of lead poisoning (*12,13*). The substrate of ALAD, δ-aminolevulinate (ALA), which accumulates in lead poisoning, has been shown to be capable of acting as a neuropathogenic agent (*11,14–19*). In fact, the clinical features of acute lead poisoning closely resemble those of the acute attacks in acute intermittent porphyria (AIP), during which ALA accumulates. AIP is a dominant porphyria due to half-normal levels of the third enzyme in the heme biosynthesis pathway. Furthermore, affected individuals with the recessive ALAD-deficient porphyria manifest chronic neurologic manifestations (*20–22*). Finally, asymptomatic heterozygotes for the latter porphyria develop acute lead poisoning when exposed to low levels of lead (*23,24*).

Three distinct charge isozymes, designated ALAD 1–1, 1–2, and 2–2 (*25,26*), result from the expression of two common alleles, designated $ALAD^1$ and $ALAD^2$, with gene frequencies of 0.9 and 0.1, respectively, in several Caucasian populations (*25–28*). The existence of this frequent polymorphism in a gene whose product was implicated in the pathogenesis of lead toxicity suggested the potential for a genetically determined differential susceptibility. We determined the ALAD isozyme types of 1278 blood samples from children subjected to low-level environmental lead exposure in New York City (*28,29*). The blood lead level, measured by the New York City Lead Screening Program, and the ALAD isozyme phenotype were determined in a double-blind fashion. Blood-lead levels and isozyme phenotypes were also determined for a population of 202 lead workers in a factory in Germany (*30*). Analysis of both populations (*29*) revealed that individuals carrying the $ALAD^2$ allele were at significantly greater risk of lead poisoning ($p<0.0001$ and $p<0.004$, respectively), with individuals carrying the $ALAD^2$ allele having about 10 μg/dl higher levels of blood lead than similarly exposed individuals who were homozygous for $ALAD^1$ allele. Human $ALAD^1$ and $ALAD^2$ cDNAs have been cloned and sequenced in our laboratory (*31,32*), and a simple polymerase chain reaction (PCR) test, based on different cleavage patterns of the two alleles by MspI, has been developed for genotyping ALAD alleles of individuals in lead-exposed populations (*32*). The present status of human studies of the contribution of ALAD genotype to lead poisoning is detailed below in the Results and Discussion section. Of special interest will be not only the acute measure of erythrocyte lead but

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also the chronic measures of dentine or bone lead.

The results reported above are consistent with the testable hypothesis that the $ALAD^2$ subunit binds lead more effectively, with similarly exposed $ALAD^2$ heterozygotes and homozygotes having higher blood lead concentrations and possibly higher lead body burden than $ALAD^1$ homozygotes. We have developed an expression system that allows production of large quantities of human ALAD 1–1 and ALAD 2–2 in *Escherichia coli* (Kaya AH and Desnick RJ, unpublished results). The present status of biochemical studies of the contribution of these ALAD isozymes to lead poisoning is detailed in the Results and Discussion section. Of particular interest will be the competition between lead and zinc for binding to the isozymes.

The human genomic sequence containing ALAD has been determined (15,913 bp, accession number X64467) in both orientations (*33*). The gene encodes 13 exons: 11 coding exons—exons 2 to 12—and 2 alternative noncoding exons—1A and 1B. The promoter with exon 1A was found to function in all cells, while the promotor of exon 1B functions exclusively in erythroid cells. The Results and Discussion section proposes mouse model experiments of lead tissue distribution, based either on published data showing variation in murine ALAD gene dose in various mouse strains (*34*) or on production of transgenic mice carrying either $ALAD^1$ or $ALAD^2$ human minigenes with promoters for either exon 1A or 1B. Of special interest will be the acute measure of erythrocyte lead, the chronic measure of bone lead, and the pathophysiologically important distribution of lead into brain.

Materials and Methods

Human Studies

Populations. To date only two populations have been studied (double-blind) for blood lead and ALAD phenotype (*29*). One group consisted of 202 male workers who were exposed to different amounts of lead for different periods of time in a German factory. The other group consisted of 1278 children from lower socioeconomic neighborhoods in New York City; at least 27 and 20% were Black and Hispanic, respectively. They had initially been screened for possible lead poisoning by the New York City Department of Health and found to have elevated free erythrocyte protoporphyrin (FEP) levels.

Additional collaborations have been established to study ALAD genotypes by PCR and MspI cleavage analysis in populations in which a chronic measure of lead exposure is available. H Hu, D Bellinger (Harvard), H Needham (Pittsburgh): chronic measure, dentine of deciduous teeth. B Fowler, E Silbergeld (University of Maryland): acute measure, blood lead; chronic measure, bone lead. DR Chettle (McMaster), L Cerhardsson, S Skerfving (Lund), V Englyst (Boliden Metall AB): acute measure, blood lead history; chronic measure, bone lead. A Todd and P Landrigan (Mount Sinai, New York): acute measure, blood lead; chronic measure, bone lead.

PCR. ALAD genotyping by PCR is carried out as previously described (*32*).

ALAD 1-1 and ALAD 2-2 Biochemistry

Expression Vectors. $ALAD^1$ and $ALAD^2$ cDNAs were ligated into a pUC19 vector with a linker such that the inducible-galactosidase promoter encoded a cocistronic message encoding a short polypeptide followed by the complete human ALAD polypeptide (*35*). The construct contained the sequence AAGCTTGGAGGAATTCTAACGCATG from the pUC19 HindIII (AAGCTT) site to the ALAD initiation codon (ATG), with TAA being the terminator for the short polypeptide and AGGAA being a ribosome binding site. The vectors were cloned in *E. coli* LC137, a lon- and htp- strain, and the inserts were completely sequenced.

Recombinant Human ALAD 1–1 and ALAD 2–2 Isozymes. Authentic, soluble, and biologically active human ALAD 1–1 and ALAD 2–2 were synthesized. The ALAD enzymatic activity was 0.7 U/mg protein, over 1000 times that found in human erythrocytes (0.45 mU/mg protein). The recombinant protein was identical to human erythrocyte protein by sedimentation velocity, polyacrylamide gel electrophoresis mobility, and Western blotting (Kaya AH and Desnick RJ, unpublished results). Purification of ALAD 1–1 and ALAD 2–2 follows the method of Anderson and Desnick (*36*).

Animal Model Studies

The 15,913 base pairs of human genomic sequence containing $ALAD^1$ were determined in both orientations (HSALADG, accession number X64467 (*33*). The analysis of the genome structure of human ALAD, necessary for constructing transgenic mice expressing human ALAD, is illustrated in Figure 1.

Results and Discussion

Human Studies

The results of the two completed studies demonstrating a relationship between blood lead levels and ALAD phenotype (*29*) are summarized in Table 1. In the 40 to 60 percentile group for blood lead in each phenotype group, individuals with the $ALAD^2$ allele (phenotypes ALAD 1–2 and ALAD 2–2) have of the order of 10 μg/dl greater blood lead than similarly exposed individuals homozygous for the $ALAD^1$ allele (phenotype ALAD 1–1). The German lead workers (FRG) showed the expected $ALAD^2$ gene frequency of about 0.1 for a Caucasian population. The children from New York City showed an $ALAD^2$ allele frequency of 0.06, consistent with inclusion of approximately 30% of children of African ancestry. No $ALAD^2$ alleles were detected in one African population (*27*). The results were not changed in a reanalysis excluding these children.

Table 1. Summary of statistics on the correlation of ALAD phenotype and blood lead.

Population	Percentile	Phenotype ALAD 1–1 Blood lead, μg/dl	ALAD 1–2 and ALAD 2–2 Blood lead, μg/dl
USA			
Number analyzed		1136	142
	40	12	20
	50	15	26
	60	18	32
	Mean ± SEM	19.5 ± 0.3	27.1 ± 1.3
FRG			
Number analyzed		160	42
	40	31	41
	50	35	44
	60	40	53
	Mean ± SEM	38.4 ± 1.3	47.0 ± 2.8

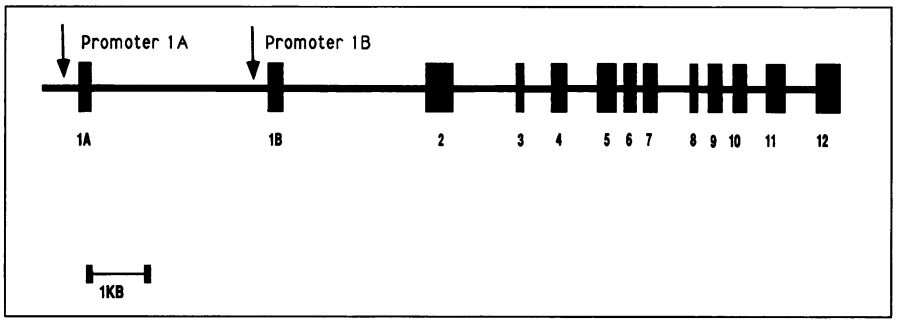

Figure 1. ALAD gene structure. Numbered exons are shown as large blocks. Promoter regions for noncoding exons 1A and 1B are indicated.

The FRG population was unselected, consisting of all participating lead workers at a factory site. The USA population consisted of children whose FEP levels on initial screening suggested the possibility of lead poisoning. FEP levels are known to be affected by other variables, including iron status (*36*). Although the identical dependence of blood lead level on ALAD isozyme phenotype for both populations argues against any bias introduced by the FEP prescreening of the USA population, additional population studies of ALAD genotype (and hence phenotype) and blood lead are needed to prove the $ALAD^2$ to be a lead susceptibility allele. Blood lead and ALAD genotype studies are in planning or in progress in populations 2 to 4 (Materials and Methods), and we welcome additional collaborative arrangements.

Blood lead is an acute measure of lead exposure. For example, there was insignificant correlation between time on the job and blood-lead level in the FRG population. If we accept the hypothesis that the ALAD 2 polypeptide binds lead tighter than the ALAD 1 polypeptide, the effect of this enhanced binding of lead on the eventual tissue distribution of lead cannot be predicted. In one scenario, ALAD 2, which is present at much higher concentrations in blood than in other tissues, could compete with lead binding proteins in brain or other organs and reduce pathology. On the other hand, increased inhibition of heme synthesis in tissues with low levels of ALAD could increase pathology. An important contribution to understanding the lead distribution problem would be to correlate instantaneous blood lead levels and lead body burden.

Integrated measures of lead exposure include lead in dentine of deciduous teeth of children and bone lead in both children and adults. Measurements of ALAD genotype together with integrated measures of lead exposure are in planning or in progress in populations 1 to 4 (Materials and Methods). The populations are listed in increasing order of numbers of subjects. Because of the low frequency of $ALAD^2$ even in Caucasian populations, the populations of lead-exposed individuals on whom integrated measures are performed must be quite large to lead to highly informative data; not even the largest of the populations identified to date is ideal. Once again, we invite collaborative arrangements.

ALAD 1-1 and ALAD 2-2 Biochemistry

The argument that erythrocyte ALAD binds a substantial fraction of the blood lead is outlined in Table 2. A unit (U) of ALAD is defined as the quantity of enzyme producing 1 nmole porphobilinogen (PBG) per hour at 37° C. The calculation includes the ALAD activity in human erythrocyte lysate (*38*) of approximately 4.7 U/mg protein (mostly hemoglobin) and the turnover number of 11 mole/min/mole ALAD subunit (*36*). The conclusion is that at least 10 μg/dl lead is bound to ALAD at 25 μg/dl total blood lead. This minimum estimate is based on ALAD enzymatic activity, which decreases throughout the 120-day life-span of erythrocytes. Additional lead could be bound to inactive ALAD. Thus the difference in observed blood lead values for individuals with different ALAD phenotype could be explained by the hypothesis that ALAD 2 subunits bind lead more avidly than ALAD 1 subunits.

Further support for this hypothesis could be obtained by comparison of the lead binding properties of the purified ALAD 1–1 and 2–2 proteins. To accomplish this objective, milligram quantities of the isozymes ALAD 1–1 and ALAD 2–2 would be needed, and the only practical source would be recombinant enzymes. Previous studies of the activities of the erythrocyte ALAD 1–1 or ALAD 2–2 isozymes have failed to detect a difference [(*25,30*), Ostasiewicz and Desnick, unpublished results], but these studies were carried out either with unpurified or with limited quantities of purified ALAD 2–2 isozyme. As described in Materials and Methods, our efforts to date have been directed toward developing an efficient systems for expression of recombinant human ALAD 1–1 and ALAD 2–2 in *E. coli*. Now that these recombinant human ALAD isozymes are in hand, we are undertaking a systematic examination of the competitive binding of lead and zinc and the effect of lead binding on ALAD enzymatic activity.

Animal Model Studies

To understand the physiologic consequences of the increased blood lead observed in individuals with the $ALAD^2$ allele, we must understand the effect of ALAD isozyme phenotype on the distribution of lead to other organs, including kidney, where specific metal-binding proteins mediate the effect of lead on ALAD, bone—the ultimate repository of much of the lead—and especially brain. Mice are especially sensitive to the effects of lead, and lead binding in various organ systems is readily determined. Furthermore, uninhibited erythrocyte ALAD activity is proportional to gene dose in commercially available mouse strains carrying one (C57BL/6), two (C58), or three (DBA/2) copies of the murine ALAD gene per haploid genome (*33*). Thus we are investigating these mice to establish the effect of ALAD concentration, especially in serum, on the pharmacokinetics of lead tissue distribution. These studies will be the basis for measurement and analysis in the more important animal model system based on expression of human ALAD alleles in transgenic mice.

Table 2. Lead binding to erythrocyte ALAD and blood lead.

ALAD
Erythrocyte ALAD activity = 4.7 nmole PBG/hr/mg lysate protein (*38*)
Reference: Whole blood contains 155 mg hemoglobin/ml
Thus, erythrocyte ALAD activity ≥ 12 nmole PBG/min/ml whole blood
Turnover number = 11 nmole PBG/min/nmole ALAD subunit (*35*)
ALAD Subunits ≥ 1 nmole/ml whole blood
Lead
1 nmole/ml = 21 μg Pb/dl
ALAD inhibition by lead
Inhibition is without threshold
Approximately 50% inhibited at 25 μg Pb/dl
Conclusion
> 10 μg Pb/dl bound to ALAD at 25 μg/dl blood lead

As described in Materials and Methods, our efforts to date have been directed toward analysis of the human ALAD gene with particular emphasis on the promoter regions. The structure of the human ALAD gene is shown in Figure 1. Expression analysis of the two promoters reveals that in both cases a DNA sequence of less than 400 base pairs produces as much product as longer constructs and exhibits tissue specificity. Thus, four minigenes are being constructed containing either exons 2 to12 of $ALAD^1$ or $ALAD^2$ and either exon 1A or exon 1B with their corresponding promoters. We propose to construct transgenic mice expressing similar numbers of copies of either the human $ALAD^1$ or $ALAD^2$ allele. The analysis of lead-exposed animals will be directed toward the study of the acute measure of erythrocyte lead, the chronic measure of bone lead, and the pathophysiologically important distribution of lead into brain. In summary, we are taking advantage of the tools of molecular biology to study the ecogenetics of lead poisoning. Our integrated tripartite approach is to study ALAD allele frequency in lead-exposed individuals by PCR, ALAD isozyme biochemistry with recombinant proteins, and the effect of human ALAD alleles on lead pharmacokinetics in transgenic mice.

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